Overview of FIND methodology for immunoassay evaluations

Introduction:
SARS-CoV-2-specific antibodies are part of the immune response to infection, and may be detected during the early, late, convalescent, and post-recovery phases of disease. They are most useful for identifying recent or prior infection. SARS-CoV-2 antigens are shed as the virus replicates during active infection, and thus their detection can be used to diagnose current infection.

About the evaluations:
1. Antibody tests: We are conducting retrospective, multicentre diagnostic evaluation studies of COVID-19 serological assays using archived, frozen serum/plasma. The index tests include novel immunoassays (RDTs or manual ELISAs) that detect antibodies specific to recombinant SARS-CoV-2 proteins, including IgA, IgM, IgG or a combination thereof. Each lateral flow format index test is being evaluated at more than one site, whereas each ELISA is being evaluated at minimally one site. For lateral flow assays, two independent operators at each site interpreted the test results, with discrepant results interpreted by a third operator. All operators were blind to the reference result. We are presenting site-specific data and combined data across sites: each site tested at least 100 positive samples and 150 negative samples.

2. Antigen tests: We are conducting prospective diagnostic evaluation studies across multiple, independent sites to determine the accuracy of COVID-19 antigen RDTs. The index tests include novel lateral flow format tests that detect recombinant SARS-CoV-2 antigens. Interim analyses are performed at 25% and 50% enrolment and the evaluation is stopped if tests do not meet 95% specificity.

Analysis:
1. Antibody tests: The estimates of sensitivity and specificity were calculated by fitting a generalized linear mixed-effect model accounting for the difference between sites and/or time-from-symptoms-onset groups. To fit the model we used the R function glmer with binomial family (logit model link function) including as random effects the site and the time-from-symptoms-onset. Two different models were used, based on two possible outcomes:

   - estimates from data from all sites, for a specific time-from-symptoms-onset group: generalized linear mixed effect model with intercept as single fixed effect, with site as random effect;
estimates from data for all sites, grouping all time-from-symptoms-onset groups: generalized linear mixed effect model with intercept as single fixed effect, with site as random effect and time from symptom onset group nested within the site.

For tests that measure multiple isotypes separately, sensitivity and specificity were calculated for each antibody isotype (e.g. IgM, IgG, IgA, and total Ig, as applicable) separately and were calculated in a combined manner, where a positive result for any isotype was interpreted as a positive test result and a negative result meant that a sample tested negative for all isotypes that could be detected by the assay. All tests were performed according to the manufacturer’s instructions for use.

**Limitations:**
Samples were purposefully (not randomly) selected for the antibody evaluations, and samples for the antigen evaluations were collected from individuals suspected to have COVID-19 according to national policy but may not capture the full spectrum of disease, therefore sensitivity and specificity estimates may not be indicative of the real-world performance of these tests in all intended use settings. Sample panel composition was different across sites, though inclusion/exclusion criteria were the same, introducing variability. Storage conditions and transit times were often prolonged and may have impacted test integrity and performance.